

THE ANALYST.

APRIL, 1895.

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS.

The monthly meeting of the Society was held the evening of Wednesday, March 6, at the Chemical Society's Rooms, Burlington House, the President (Dr. Stevenson) occupying the chair.

The minutes of the previous meeting were read and confirmed.

Mr. D. J. O'Mahony, Public Analyst for Cork, was elected a member of the Society.

Dr. Dupré read the following paper:

NOTE ON THE CHEMICAL AND BACTERIOLOGICAL EXAMINATION OF WATER, WITH REMARKS ON THE FEVER EPIDEMIC AT WORTHING IN 1893.

By A. DUPRÉ, Ph.D., F.R.S., F.I.C., Lecturer on Chemistry at the Westminster Hospital Medical School.

It has long been known that certain diseases, as cholera or typhoid fever, were liable to be spread by water which had been contaminated by discharges coming from persons suffering from these disorders. It is, however, only within comparatively speaking recent years that this liability of spreading disease was traced definitely to the presence of certain living organisms occurring in the discharges coming from persons suffering from these disorders. This having once been recognised, the conclusion was not perhaps unnaturally arrived at that a water could not be condemned as dangerous unless it contained some of these pathogenic organisms; and chemical analysis, being unable to detect these, was condemned as useless. It is because I firmly believe that such condemnation is unwarranted that I venture to bring this subject before you this evening. I need say but little about methods of analysis, but I cannot help drawing attention to the recent remarkable demonstrations of the difficulty connected with the carrying out of the organic carbon and nitrogen process devised by Professors Frankland and Armstrong. Those who have followed the analyses published monthly in the report of the official water examiner must have noticed that while Professor Frankland almost invariably gave the organic carbon higher than did Professors Odling, Crookes, and Tidy, the latter, almost as invariably, gave the organic nitrogen higher than Professor Frankland. The result was that the ratio between organic carbon and organic nitrogen, on which at one time Professor Frankland pinned his faith in judging of the quality of a water was not infrequently twice as high in the one series as in the other. Here, then, we have

on the one hand one of the inventors of the process, a chemist of the highest reputation, whose experience of this method is certainly unrivalled, and on the other two of our foremost chemists, and the most enthusiastic convert to the process, differing month after month, and year after year, in this remarkable manner. What confidence, I ask, can we place after this in the process in the hands of chemists who cannot claim one-hundredth part of the experience of these observers? Quite lately a change has come—in consequence, we are told, of some hints given by Professor Frankland—and the two series of analyses now agree very fairly. If Professors Dewar and Crookes are not above taking a hint from another quarter, I would advise them to discontinue their old unscientific method of estimating the oxygen absorbed from permanganate, and adopt the method now, I believe, very generally employed; namely, the use of stoppered bottles, a temperature of 80° F., and four hours' action. For the purpose of this paper it does not, however, matter what particular method of analysis is adopted, provided it is carried out with care and skill. I will only add on this subject that in my opinion no judgment on any water should ever be formed on one or two analytical features only, but that as many points as possible likely to throw light on the subject should be investigated. For my part, I have for many years been in the habit of giving colour, smell, appearance (clear or turbid), deposit and character of deposit, nitrous acid, phosphoric acid, hardness before and after boiling, total dry residue, colour of the same and behaviour on ignition, oxygen absorbed from permanganate in four hours, sometimes also in fifteen minutes, chlorine, nitric acid, ammonia, albuminoid ammonia, and, only if specially desired, organic carbon and nitrogen. With the help of these data a just conclusion may, I think, be nearly always arrived at.

The next point is, What does such a chemical analysis teach us? It does not, as before stated, show us the presence or absence of pathogenic organisms, or, indeed, of any living organism; and in this respect it is, of course, defective. (Examination of the deposit by the microscope often teaches us a great deal, but is not a chemical examination). But it does show us, in ninety-nine cases out of a hundred, whether the water is polluted by sewage or not, particularly if we adopt the plan proposed by Mr. Hehner and myself—viz., comparing the water under examination with one from the same district and formation which the surroundings show to be unpolluted. No doubt, if a water should be contaminated by a small proportion of, say, a typhoid stool, analysis may not be able to detect this pollution. Such a case, however, very rarely happens, except with artificially-prepared samples; but if it should occur naturally, the analyst would most likely overlook it. In nearly all cases, however, a polluted water will contain the sewage from many healthy persons for that from one person suffering from typhoid, and although the latter by itself could not be detected, the sewage from the larger number of healthy persons can and will be detected. The fact of such pollution having taken place will—and this is a most important point—be detected, however perfect may have been the natural or artificial filtration, and it will of course be detected, whether any pathogenic organisms are present or not.

Now we know that a water polluted by sewage, although at the time of analysis no infected sewage was present, may at any time become dangerous if anyone of the

persons within the drainage area of the well should suffer from typhoid or cholera. The chemist is, therefore, able to anticipate what may happen in the future; and as no one will pretend that a sewage-contaminated water, even if free from pathogenic organisms, is wholesome, the chemist, by advising the closing up of a well or the abandonment of a watercourse, will close, so to speak, the stable-door before the steed is stolen. To put, then, the case of the chemist shortly:

While unable to detect the presence of pathogenic organisms, he is, at any rate in the great majority of cases, able to detect the presence of sewage pollution. He is thereby able to anticipate what may happen in the future, and by timely advice prevent an outbreak of disease.

What, now, can the bacteriologist accomplish? He is, in some cases at least, able to detect the presence of pathogenic organisms in a water, but only *after* the water has become infected. When an outbreak has occurred, he will furnish the final proof that it has been caused by the water—a fact which, quite irrespective of such proof, would in nine cases out of ten have been demonstrated by altogether independent investigation. The bacteriologist, of course, cannot find pathogenic organisms where they are not present, and has not, at least not in the same degree, the power of anticipating the future possessed by the chemist. He is able to close the stable-door, for conclusive reasons, but only *after*, not *before*, the steed has been stolen. I shall of course be told that in a sewage-polluted water the bacteriologist is able to detect numerous organisms non-pathogenic, but, like the bacillus coli, for example, characteristic of sewage. I answer, this may be so; but you condemned chemical analysis on the ground that it cannot detect pathogenic organisms, and you cannot run with the fox and hunt with the hounds. If you acknowledge that sewage-polluted water is not fit for human consumption, whether specially infected or not, you place yourself in the same position as the chemist, and the question will have to be decided, how can sewage pollution be most certainly detected, chemically or biologically? Do not, however, condemn analysis on the ground that it cannot detect pathogenic organisms, and then presume to judge a water, not by the presence or absence of pathogenic organisms, but by the mere number of the micro-organisms present. The proposal to condemn a water because it contains, say, more than a hundred micro-organisms per cubic centimetre appears to me to be simply nonsense. Take a deep well water, Kent water, for example, which in many instances, as it comes from the well, contains as few as ten organisms per cubic centimetre, or even less. Keep this same water for a day or two in moderately warm weather, and it contains thousands in the same volume. Are we to be told that Kent water is an admirable drinking water as it comes from the well, and absolutely unfit for drinking one or two days after?

By means of biological examination we are probably better able to detect pollution in a water contaminated by unfiltered sewage; but this kind of pollution is, in well waters at least, comparatively speaking, a rare occurrence. In most cases the sewage enters the well after filtration through more or less soil, in which many of the organisms remain behind. Experiments with artificially-polluted waters, in which the pollution is brought about by the addition of a small quantity of an infected stool, and without its proper complement of urine, are therefore greatly misleading.

They unduly favour biological examination as compared with chemical analysis. It would be extremely easy to prepare a water highly polluted by sewage, which, on biological examination, would have to be pronounced absolutely free from contamination, but in which chemical analysis would reveal the presence of sewage with the greatest ease. Would biologists consider such an artificially-prepared water a fair sample to judge between biological and chemical examination? I am, of course, aware that there is at least one case on record in which, in all human probability, a single discharge from a person suffering from typhoid, polluting a well, caused a serious outbreak of typhoid. (We shall presently see that, according to Dr. Kelly, the typhoid outbreak at Worthing two years ago was a case of a similar character.) In such a case bacteriological examination might possibly have succeeded in tracing the mischief to the well, while analysis would probably have failed. But biology would only have succeeded when it was too late to prevent mischief. Moreover, such cases are necessarily rare, and never ought to occur; they cannot fairly be brought forward as proving the general superiority of biological examination over chemical analysis. I do not wish to be regarded as hostile to the bacteriological examination of water, but I do not believe that it can replace chemical analysis. In many cases biological examination is, of course, of the highest value. Take the case of our London water-supply. We know that the Thames is largely polluted by sewage, which in many cases is infected sewage, and we do not as yet know sufficient of the life-history of these organisms to be justified in affirming that none will ever reach the intake of the waterworks, and find their way on to the filter-beds. Although, as far as I know, none have ever been detected there, their possible presence must be accepted. The safety of the supply depends, therefore, on the efficiency of the filtration. If, then, it can be shown that filtration is able to remove 99 per cent. of all the micro-organisms present, we are justified in concluding that the chance of pathogenic organisms finding their way into the filtered water is but slight. To judge the efficiency or otherwise of the filtration of such a water, biological examination is certainly a far more effective guide than analysis. But this case also shows the weakness of biological examination. On the strength of it, we are forced to pronounce the water to be pure and free from pollution, whereas the chemist has no difficulty in pronouncing it sewage polluted. Armed with this knowledge, he is able to say: "This water is polluted by sewage, and although not in its present condition injurious, it may at any time, through a breakdown of the filtering arrangements, become dangerous."

Biological examination of water is yet in its infancy; but, judging by the great strides in advance it has made during the last few years, we may confidently look forward to further improvements in the near future. Nevertheless, it will not, in my opinion, ever be able to replace chemical analysis.

Let me draw attention, in passing, to the conveniently small measure adopted by bacteriologists to record their results; namely, the number of colonies yielded by 1 cubic centimetre of water. Now, one organism per cubic centimetre means 4,543 per gallon, or about 300 in an ordinary tumblerful of water. Koch's limit of 100 colonies per cubic centimetre means, therefore, 453,600 per gallon, or 30,000 per tumbler. How beautifully free from impurity would most water, or even sewage,

appear if we stated our analytical results in parts per cubic centimetre, instead of in parts per 100,000, per million, or in grains per gallon. Now, inasmuch as nobody can tell, at present, how small a number of pathogenic organisms may, say under conditions most favourable to their action, produce serious effects, this statement of organisms per cubic centimetre seems very unsatisfactory, seeing that many of us are in the habit of frequently drinking several pints of water a day. No doubt if the bacteriologist finds pathogenic organisms in a water, that water stands condemned, whatever its other qualities may be; but does the converse hold good? Are we justified in pronouncing a water as perfectly safe in which the bacteriologist has been unable to find any such organisms? Can we be sure that the cubic centimetre, or the litre, or the two litres he has examined contained their proper proportion of all the various organisms present in a large bulk of water? Do we know enough of the conditions of existence of these organisms to be justified in such a conclusion? What would be thought of a man who, if he, say, in ten cubic yards of sea-water found no fish, were to pronounce that there are no fish in the sea? or who, when he found a certain number of herrings in ten cubic yards in the midst of a shoal of herrings, would take this as a basis for calculating the number of herrings in the sea? The conclusion, then, that I have arrived at is that each of these two methods of examination has its strong and its weak points, and that neither of them can fully replace the other. They should, if possible, be used in conjunction with each other; but if one kind of examination only can be done, this one should be chemical analysis.

I cannot conclude without making some remarks on the outbreak of enteric fever in Worthing in the spring and summer of 1893, as it offers a good example of the manner in which chemical analysis is often contrasted with bacteriological examinations, to the disadvantage of the former.

Dr. Theodore Thomson says (p. 17 of his report): "Several chemical analyses of 'Worthing' water, before, as well as after, the date on which the new supply was utilized, are given in Appendix B, and from these it will be seen that, as not infrequently happens, chemistry failed to detect any definite impurity in the water. But the result of bacterioscopic examinations, given in Appendix C, furnishes conclusive evidence of contamination of this supply by faecal matter. Let us see how the case really stands. The first case of enteric fever at Worthing was notified on May 3 (as subsequently ascertained, the first attack had occurred on April 16, between which date and May 2 fifteen more persons were attacked). The first sample of water sent to me was drawn on May 2 from just below the surface of the water in the well (well C), while the second sample was taken on May 6 from a depth of 57 feet below the level of the ground, 30 feet below the water-level, and $15\frac{1}{2}$ feet from the bottom of the well. I reported both samples as unpolluted by sewage. At the time I was entirely ignorant of what had happened at Worthing; but even now, with a full knowledge of this, I am still strongly of opinion that these samples, particularly that of May 6, were not polluted by sewage. The latter sample is chemically purer than average Kent waterworks water.

On May 15 another sample of water was taken from the same well (well C), but this time from the bottom, and close to the new heading, through which the pollution was supposed to enter the well. This sample was examined by Professor

Crookshanks, who found it to contain 118,000 bacteria per cubic centimetre. In his report no mention is made either of bacillus coli or the enteric fever bacillus, and as the examination was made with a full knowledge of what had happened at Worthing, we must assume that none were present. On June 5 two more samples were taken from the bottom of well C. One sample was sent to Professor Crookshanks, the other to Professor Millar Thomson. Professor Crookshanks sums up his report as follows: "The water-sample of June 5, 1893, ranks, from the bacteriological analysis, as very pure water." Professor M. Thomson's analysis shows the water to have been, on the whole, not quite as pure as my sample of May 6, but he reports: "On the whole, the chemical analysis of the water sent does not point to the water being unfit for drinking purposes." Here, then, we have three chemical analyses and two bacteriological examinations, none of which point to sewage contamination, the second bacteriological examination being, in fact, highly favourable. Let me repeat here that my second sample—the most important one—was taken 15½ feet from the bottom of the well, and 9½ feet above the crown of the suspected heading. It was, therefore, in all probability, purer than a sample taken from the bottom would have been. Why are these two bacteriological examinations, made by an eminent observer, not mentioned in Dr. Th. Thomson's report? Where, I ask, is the failure of chemical analysis as compared with bacteriological examination, for both methods point to the same conclusion?

On July 26 a further sample of water was taken from well C and sent to Dr. Klein. The depth from which the sample was taken is, unfortunately, not given, but it was probably taken from the bottom of the well. In this sample Dr. Klein detected, apparently, five bacillus coli in the particulate residue from 2,500 cubic centimetre of water, driven through a Berkefeld filter. No enteric fever bacilli were detected in this sample.

On August 2 another sample of water was taken at the Worthing Waterworks, this time drop by drop during twelve hours, from the rising main, through which the water from all the wells, headings, etc., passed. In 1,500 c.c. of this water Dr. Klein detected an abundance of bacillus coli, and three organisms, two on one plate and one on another, "which on sub-culture presented morphologically, as well as culturally, all the characters of the enteric fever bacillus." Observe, Dr. Klein does not say that what he found was actually the enteric fever bacillus, and he was evidently alive to the fact that it might have been some other organism, otherwise why this caution? If a chemist were to state in his report that he found something which gave the reaction of, say, nitric acid, but did not say that it was nitric acid, we should all know what to think of such a statement.

Before finishing this part of the case, it is necessary to say that in Dr. Th. Thomson's report, p. 16, it is clearly shown that under ordinary conditions no sewage would find its way to the near neighbourhood of the heading through which the pollution is supposed to have entered the well, but that when a heavy rainfall coincided with a tide-locked condition of the sewers, some sewage, by being backed up along an old disused overflow pipe, might find its way there. Now, during March and April, 1893, the rainfall at Worthing was 0.33 inches and 0.08 inches respectively, and for seven weeks prior to May 6 practically no rain at all fell. On

the other hand, during June and July the rainfall amounted to 0·58 and 4·95 inches respectively, of which no less than 3·05 inches fell within ten days of June 26 and 0·41 inches on the 26th itself. Where, I ask, is the justification for comparing samples taken on May 2 and 6, at a time of little or no rainfall, and an epidemic only just beginning, with the samples of June 26 and August 2, after heavy rainfall and an epidemic only just past its height, after having run its course for over four months?

If such a conjunction of favourable circumstances is necessary to enable a bacteriologist to detect the presence of three organisms which on subculture presented morphologically and culturally all the characters of the enteric fever bacillus, all I can say is, save us from the bacteriologist!

But the case against bacteriology is not by any means exhausted with regard to this Worthing outbreak.

So far I have only dealt with the outbreak at Worthing and other places, the water-supply of which was taken from the Worthing waterworks. There was, however, a similar outbreak at West Worthing, which has a separate supply. This outbreak was also traced by Dr. Th. Thomson to the water-supply, with this difference: that he believes, from the localization of the outbreak in certain districts of West Worthing, that it was due to local pollution of the water in the mains of these districts, and not to pollution of the water at the waterworks. Here, then, was a fine field for the bacteriologist, who, moreover, as no chemist was employed, had the field all to himself. The result was that Dr. Klein was unable to detect the bacillus coli or the bacillus of enteric fever in either the water taken drop by drop during twelve hours from the rising main at the waterworks, or in the water taken from a tap in direct communication with the water-main in one of the worst stricken streets of West Worthing. It would be highly interesting if we knew what a chemist could have done. What does Dr. Th. Thomson say of this failure of his favourite instrument for water examination? After some remarks I need not quote here, he says: "For specific organisms are usually present in water in small amounts only, and could readily be absent in the small samples selected for examination." Precisely so; this is exactly my view. Any comment of mine is unnecessary. But it is advisable to point out that here we have not only complete failure to detect the pathogenic organisms which must have been present in the supply, but also a total failure to detect the bacillus coli, the organism by help of which mainly the chemist was supposed to have been vanquished. I ask again, where is the justification for extolling bacteriology at the expense of chemical analysis?

In conclusion, it may not be uninteresting to state that, in the opinion of Dr. Kelly, the Medical Officer for Worthing, the outbreak was not due to any sewage pollution of the water in the ordinary sense, but was due, like the case at Caterham, to the misbehaviour of some of the workmen working in the new heading shortly before the outbreak began. Dr. Kelly wrote to me on October 4, 1894: "Chemistry failed to help us because, as a matter of fact, no sewage entered into our new water-supply." My own opinion is, that the greater portion of the epidemic was due to pollution of the water in the mains of the town of Worthing just as in the case of West Worthing.

Dr. Thresh then read the following paper :

THE INTERPRETATION OF THE RESULTS OBTAINED UPON THE
CHEMICAL AND BACTERIOLOGICAL EXAMINATION OF POTABLE
WATERS.

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Medical Officer of Health to the Essex County Council.

PRIOR to obtaining a qualification in medicine and in public health, and to becoming a Medical Officer of Health, I had had a considerable experience in the analysis of potable waters, and, like most other analysts, I was accustomed to declare a water good and safe, or impure and dangerous, according to the results of my analyses, basing my opinion upon comparison with standards arbitrarily set up either by analysts of repute, or by learned societies who had given their attention to this subject. Under the conditions of my appointment as Medical Officer of Health, the analysis of drinking-waters formed part of my duties, and as such has led to my having examined probably 1,000 samples of water during the last five or six years.

I was not long in discovering, what other Medical Officers of Health had previously discovered, that my analytical results often directly contradicted the results of my observations as to the source of the water, and its effects upon the health of those who used it. My standards condemned waters as impure and unsafe which had never been known to produce any ill effect, and which came from sources which, so far as I could discover, were perfectly safe and free from any injurious contamination. In fact, if I were to condemn every source of supply in my districts which yielded an excess, for example, of albuminoid ammonia and nitrates, I should leave many localities entirely without a supply. On the other hand, waters which I have had the gravest reasons for suspecting as the cause of disease, yielded on analysis much more favourable results than others not suspected, and yet upon close observation such waters were found either actually contaminated with sewage or liable to intermittent pollution.

Further experience has also unfortunately brought me in direct conflict with analysts who have differed from me in opinion as to the quality of a water. The analyst judging from the results of his examination only; I basing my opinion not only on the analysis, but also upon an examination of the source of supply, to ascertain the possibility or otherwise of its contamination. My experience, I am sorry to say, is that of other health officers, and it is my desire to avoid such conflict of opinions in future that has induced me to read this paper. Let it be understood that I am not now referring to difference of opinion arising from crass ignorance, as in a case recently submitted to me, in which a deep well water containing a considerable amount of chlorides was stated to be probably polluted with urine. Had the analyst known anything of the nature of the water derived from the source from which this came, he would not have made such an absurd statement.

Of the many impurities which drinking-water may contain, the organic matter only is a serious source of danger, and by far the greatest risk is incurred in using waters liable to contain certain living organisms which, when introduced into the

system, are capable of producing specific disease. Of the presence or absence of such organisms chemical analysis can give us no information. The presence of organic matter may be chemically demonstrated, but inasmuch as the nature of this organic matter, whether poisonous or innocuous, is beyond the power of the analyst to reveal, it is obvious that the results of a mere chemical analysis may often be worthless or even misleading. This point cannot be too strongly emphasized, since the popular impression, shared alike by the ignorant and the learned, that a chemist, by performing a few mysterious experiments with a water in his laboratory, can pronounce at once whether it be safe or dangerous, must be dispelled. This opinion has been fostered by those analysts who do not hesitate to pass judgment upon a water merely from the results of their chemical examination, from the determination of the chlorides, nitrates, phosphates, and ammonia, of the organic carbon and nitrogen, and of the oxygen consumed, or of the ammonia derivable from the organic matter. All these factors are of more or less importance as an index of the degree of pollution, recent or remote, but their real value can in very few cases be assessed without some previous knowledge of the source of the water. The inorganic constituents can easily be determined, and whether, either in quantity or quality, these are objectionable, the chemist can safely express an opinion. But this is more medical than chemical. For our purpose, therefore, only those salts need be considered which by their presence tend to throw some light upon the source of the organic matter, always present in greater or less quantity. These are the chlorides, phosphates, nitrites, nitrates, and ammonia, and inasmuch as their determination is often of importance, the value of each may be discussed.

Chlorides.—In the great majority of instances the only chloride present is chloride of sodium or common salt; occasionally other chlorides (calcium, magnesium, etc.) are also found, especially if the water be derived from wells near the sea-coast. Rain-water always contains a trace of salt, the amount varying with the distance from the sea. Certain geological formations also are rich in salt, and waters obtained therefrom may contain considerable quantities of it. Urine, again, contains nearly 1 per cent. of salt; hence pollution with sewage will add chlorine to the water. The effluents from many manufactories, alkali works, mines, etc., are also rich in chlorine. From these various sources, therefore, the chlorides found in waters are derived. When the geological strata contain little or no salt, and there are no manufacturing or mining effluents to pollute the water, the amount of chlorides present may serve roughly as an index of the extent to which it has been contaminated by sewage.

In Massachusetts it has been found that the amount of chlorine in the surface waters and streams decreases in amount from the seaboard westward or inland. By the examination of waters from sources removed from all risk of contamination, the normal chlorine for such districts has been determined. "By placing on the map of the State the amount of chlorine normally present in its unpolluted waters, and then connecting the points of equal amounts, lines of like chlorine contents are obtained, which are called *isochlors*." From the map thus prepared the normal chlorine is found to vary from .45 grain per gallon near the coast to less than .06 in the western part of the State (Board of Health Report, 1892). Over any given area the amount of chlorine in excess of the normal, as above ascertained, can only

be due to the influence of the population discharging its sewage thereupon. Assuming that 100 persons per square mile add on an average .03 grain per gallon of chlorine to the water flowing from that area, the extent of the contamination can be approximately calculated. It must be remembered, however, that the amount of chlorine present does not necessarily signify present pollution. The organic matter which originally accompanied the salt, and which alone is deleterious, may have undergone complete oxidation and destruction, so that organically the water may be very pure, although the amount of chlorine present indicates that at one time it was excessively polluted. This fact detracts very considerably from the importance of the chlorine determination. It affords some evidence of the previous history of the water, and that is all. In insular countries the estimation of the chlorine is of even less value, since they cannot be mapped out into *isochlors*. Over limited areas, however, the normal chlorine may sometimes be ascertained, and any excess found in samples from that district will be in a measure proportionate to the present or past pollution of the water. For example, in the parish of Writtle, all the wells in which I recently examined, the normal chlorine did not exceed 2.5 grains per gallon, yet in that parish subsoil waters were found containing as much as 14.0 grains per gallon, and that this was due to past and present pollution with sewage was substantiated by the excess of other substances, especially nitrates, which, as we shall see, are also in most cases derived from the same source. Unless this normal chlorine be known, the determination of the chlorides has no value whatever. The variation in the amount of chlorine in pure surface waters from various geological formations being known, any excess over these amounts would probably point to past or present pollution, and in any case would indicate that farther investigation of the source was desirable or necessary. In shallow-well waters, even when pure, the chlorine varies so greatly in amount that it is only in rare cases, as in the one referred to above, that the determination affords any information of value. In spring waters also it is difficult to decide upon the normal chlorine of any particular formation, but if in any case the amount found greatly exceeds the average, past or present pollution is indicated. The same remark applies to deep-well waters. In several instances, when examining water from the deep wells in Essex, I have found that the chlorides present were below the normal, and have been able to prove that this was due to surface water (usually impure) having gained access to the well. If the source of the water be not known, reliance upon the chlorine estimation may lead to serious error. The possibility of the excess of chlorine being derived from manufactories or mines must also be considered before concluding that the water contains contaminating matter of animal origin.

Nitrates and Nitrites.—The combined nitrogen found in drinking-waters may be present as organic matter or in ammonia, nitrites, and nitrates. Traces of all three are found in most samples of rain-water. Nitrogenous organic matter undergoing putrefaction invariably produces ammonia, and by oxidation this ammonia is converted, by micro-organisms found in all soils, into water and nitric acid, the latter decomposing the carbonates present, and forming nitrates of soda, potash, or lime. The ammonia, however, is not apparently converted directly into nitric acid, but passes through an intermediate stage, a lower oxide of nitrogen, nitrous acid being

first formed. The Rivers Pollution Commissioners found that whilst the organic matters contained in sewage, and therefore of animal origin, yielded abundance of nitrates and nitrites by oxidation, no less than 97 per cent. of the combined nitrogen of London sewage being converted into nitrates by slow percolation through 5 feet of gravelly soil, vegetable matters yielded mere traces of these compounds. Upland surface waters "in contact only with mineral matters, or with the vegetable matter of uncultivated soil, contain, if any, mere traces of nitrogen in the form of nitrates and nitrites; but . . . as soon as the water comes in contact with cultivated land, or is polluted by the drainage from farmyards or human habitations, nitrates in abundance make their appearance." Subsoil waters derive their nitrates in part from the oxidized ammonia of rain water, in part from the slow decay of vegetable matter, and in part from sewage matters. The amount derived from the two former is almost invariably small. Vegetable matter is not highly nitrogenous, and as a rule decomposes but slowly. Animal matter, on the contrary, decomposes rapidly and yields much ammonia. Nitrates serve for the food of plants, and the active growth of vegetation may remove nearly the whole of these salts from a water. In reservoirs the nitrates decrease gradually as the vegetable organisms increase. The total combined nitrogen, therefore, in a water may at one time exist in decaying animal and vegetable matter, or in the form of ammonia; at another in the form of nitrites and nitrates, and yet again as a constituent of the protoplasm of living vegetable organisms, in which latter case it is not in solution, but merely suspended in the water. Whenever organic matter undergoes putrefaction in the absence of air or free oxygen, not only are nitrates not formed, but any nitrates present are decomposed, their oxygen being required for the formation of water and carbonic acid by combination with the carbon and hydrogen of the decomposing substances. The nitrogen appears to be set free, possibly accounting for the excessive amount of that element found in such deep-spring waters as those of Bath, Buxton, and Wildbad. In this way the small amount of nitrates found in most deep-well waters is accounted for. Such being the case, it is evident that even concentrated sewage may undergo such changes as would totally obscure its origin so far as the combined nitrogen is concerned. At first it would be contained chiefly in the dissolved animal impurities; after passing through the surface soil, it would exist chiefly in the nitrates formed by the oxidation of the organic matter; later the nitrates may be decomposed, and the nitrogen liberated, when the water would be almost or entirely free from combined nitrogen. On the other hand, certain deep-well waters, especially in the chalk, contain very considerable amounts of nitrates, which it is difficult to believe are derived from the oxidation of sewage matters. It has been suggested that these nitrates are derived from fossil remains, or from natural deposits of nitrates, or from vegetable matter; but as no proof of these statements is forthcoming, they must be received with reserve. In the eastern counties the chalk wells yield waters which in some districts are absolutely free from nitrates (south-east Essex), whilst in other districts (Norfolk) they may contain possibly as much as 1 grain of nitric nitrogen per gallon. The following may be quoted as examples:

	Nitric N. per gallon.	Depth of Well.	Authorities.
		feet.	
Stratford: Phoenix Works	·00	200	J. C. Thresh.
Wimbledon... ..	·03	200	"
Chatham Public Supply	·48	490	"
Southend	·05	900	"
Witham	·45	600	R.P.C.
Mistley: Tendring Hundred W. W. Co. .	·05	160	J. C. Thresh.
Braintree Public Supply	·02	430	T. A. Pooley.
Colchester (Donyland Brewery)	·00	305	J. C. Thresh.
Saffron Walden Public Supply	·95	46	"
Norwich	·80	About 400	"

In none of the above examples is there any possibility of recent sewage contamination.

Notwithstanding these facts, the Rivers Pollution Commissioners considered the total combined nitrogen to be an index of previous sewage contamination. They assumed that 100,000 parts of average London sewage contains 10 parts of combined nitrogen in solution. The mean amount of such nitrogen found in a large number of samples of rain-waters examined was ·032 per 100,000. After deducting this latter amount from the amount of nitrogen, in the form of nitrates, nitrites and ammonia found in 100,000 parts of a potable water, the remainder, if any, they say, "represents the nitrogen derived from oxidized animal matters, with which the water has been in contact. Thus, a sample of water which contains, in the forms of nitrates, nitrites, and ammonia, ·326 parts of nitrogen in 100,000 parts, has obtained $\cdot326 - \cdot032 = \cdot294$ part of that nitrogen from animal matters. Now, this last amount of combined nitrogen is assumed to be contained in 2,940 parts of average London sewage, and hence such a sample of water is said to exhibit 2,940 parts of previous sewage or animal contamination in 100,000 parts." The Rivers Pollution Commissioners, however, point out that, on the one hand, the nitrates may not indicate the full extent of the previous sewage pollution, since the roots of growing crops take up much of the ammonia, nitrites, and nitrates contained in polluted water, and animal matter, which decomposes without access of air, destroys nitrates; and, on the other hand, that the nitrates present may indicate 10 per cent. of previous sewage contamination in deep wells and springs, and the risk of using such waters be regarded as nil, providing surface pollution be rigidly excluded. This 10 per cent. of previous sewage contamination corresponds to one grain of nitric nitrogen per gallon.

Mr. F. Wallis Stoddart, in an excellent paper on 'The Interpretation of the Results of Water Analysis,'* describes a series of experiments in which he passed sewage containing cholera bacilli through a nitrifying bed of coarsely-powdered chalk, and found that although the organic matter in solution was completely nitrified, yet the cholera bacilli or spirilla could be detected in the effluent. From the result of his own observations and experiments, he concludes that natural waters "can at most obtain from one-tenth to two-tenths of a grain of nitrogen as nitrates per gallon from

* *Practitioner*, 1893; reprinted in *ANALYST*, xviii., 293; xix., 18, 39.

sources other than animal matter," and "that practically the whole of the nitrogen of sewage may be oxidized into nitric acid without materially diminishing the risk involved in drinking it." He urges that whenever the nitrogen as nitrates exceeds half a grain per gallon, it indicates "either dangerous proximity of the well to a source of pollution, or such easy communication with it that the distance separating the two points is no guarantee of purification." Many waters, the source of which I carefully examined, and which were collected and analysed by myself, contain more than this amount of nitric nitrogen; and I am perfectly convinced that these waters are hygienically of the highest class, and can be used without the slightest risk or danger. On the other hand, in the table given at the end of this paper, there will be found many waters containing very much less nitrogen as nitrates which have almost certainly (in most cases the proof was most conclusive) given rise to outbreaks of typhoid fever. If Mr. Stoddart's maximum of $\cdot 5$ be accepted as proof that a water is dangerous, then the public and private water-supplies of many of our healthiest districts—districts remarkably free from outbreaks of typhoid fever—must all be considered dangerous. As a matter of fact, the amount of nitrates which would condemn a water from one source may be absolutely without significance in another, all of which goes to prove, as will be shown in the sequel, that mere chemical analysis is absolutely powerless to prove that any water is of such a quality as to be incapable of producing disease amongst those who drink it.

Nitrites may result from the oxidation of ammonia, or from the reduction of nitrates, and, as it is an easily oxidizable compound, its presence indicates a condition of instability, of matter undergoing change. Usually this matter is of animal origin and derived from manure or sewage, the ammonia produced by their decomposition being in process of oxidation to nitrates. Where the soil is not sufficient in quantity, or is defective in quality, the oxidation may be incomplete, and incompletely purified and probably incompletely filtered water is the result. Usually, in such cases an excessive amount of ammonia is also present. But, though usually, this is not invariably the source of the nitrates and ammonia. Where nitrates are present the nitric acid may be reduced by contact with metals, such as iron or lead, forming the pipes in which the water is conveyed, or lining the upper portion of the well. Where such is the case, a trace of the metal can always be detected in the water. Unless this fact be borne in mind—and it often appears to be overlooked—a good and wholesome water may be classed as dangerous or polluted. Certain organisms also found in water are capable of reducing nitrates to nitrites. Still, the presence of nitrites always renders a water suspicious, and their origin should be carefully investigated.

Ammonia.—All rain-water contains this compound, as does also melted snow. The first portions of a shower, and the rain collected in the neighbourhood of towns are richest in ammonia. As an average, $\cdot 02$ grain per gallon, taken by the Rivers Pollution Commissioners, is probably fairly approximate, but the variation is very wide ($\cdot 2$ to $\cdot 01$). In passing over or through the ground the ammonia is rapidly oxidized, and by the time the water reaches a stream or the general body of subsoil water, most of it has disappeared. Rain-water stored in covered cisterns, however, usually retains its ammonia for a considerable period. In such waters, therefore, the ammonia, unless excessive, is devoid of significance. Many deep-well waters also

contain much ammonia, the origin of which has given rise to a good deal of surmise. The generally-accepted theory is that it is due to the reducing action of ferruginous sands on the nitrates present. This may be so in some cases, but my observations lead me to believe that it is often due to the reduction of the nitrates by the metal of the bore tube, pump pipe, and lining of the well. I was led to this conclusion from the fact that I found the water from one and the same well at one time quite free from ammonia, and at another containing as much as one part of ammonia per million parts of water. In the water containing ammonia I also found a very faint turbidity, which cleared up on the addition of a little acid, and gave the reactions for iron. The clear, ammonia-free water also, when stored for a time in an iron tube, became turbid, and nitrites, ammonia and iron could be detected in it. Generally, however, the ammonia found in river, spring, and well waters is derived from putrescent animal matter—that is, from manure and sewage; but before this conclusion can be safely drawn, the other possible sources must be excluded. Dr. Brown, in his *Report to the Massachusetts State Board of Health*, 1892, whilst agreeing that imperfect oxidation of sewage matter is generally the source of the ammonia, calls attention to the fact that several waters in the State free from such pollution contain a considerable amount of free ammonia. “They are all associated with iron oxide and the fungus *Crenothrix*.” Such waters are found also in many swampy regions, and in wells sunk in ferruginous river silt, and usually become turbid from the formation and deposition of oxide of iron when exposed to the air. The odour of these waters is said to be “often disagreeable from dissolved sulphuretted and carburetted hydrogen.”

Phosphates.—Phosphates are found widely distributed in rocks and soils, and traces may be dissolved by a water rich in carbonic acid. Albuminous matters, whether of animal or vegetable origin, give rise to phosphates by their decay, hence an excessive amount present in a drinking-water is held by many analysts to indicate contamination. The difficulty of determining their presence and amount is considerable, and as the value of the determination is very doubtful, few analysts pay any attention to them. They may be present in a pure water, and their absence is no proof that a water is free from pollution.

Organic Matter.—By no known process can either the quantity or quality of the organic matter in water be determined. When a known volume of water is evaporated to dryness, the weight of the residue is that of the inorganic and organic substances contained therein. When this residue is ignited the organic matter is destroyed and volatilized, and the “loss on ignition” has been regarded as approximately expressing the weight of the organic constituents. Such, however, is rarely the case, since carbonic acid may be driven off from the carbonates present, and any nitrates present will be more or less completely reduced. Moreover, some salts retain water so tenaciously that the whole is not driven off at the temperature used for drying, and this moisture is given off when the residue is ignited. For these reasons, chiefly, the “loss on ignition” cannot be depended upon as an index of the amount of organic matter present. But although the total amount of the animal and vegetable substances cannot be determined, the carbon and nitrogen therein can be ascertained by careful chemical analysis. Not only so, but the authors of the original process believed that, with certain reservations, the propor-

tion of the nitrogen to carbon indicated whether the organic materials are derived from the animal or vegetable kingdom. In fresh peaty water the Rivers Pollution Commissioners found that $N : C = 1 : 11.93$, whilst in similar waters, which had been stored for weeks or months in lakes, $N : C = 1 : 5.92$. After such water had been filtered through porous strata, $N : C = 1 : 3.26$. In fresh sewage the average of a large number of samples gave $N : C = 1 : 2.1$. Highly polluted well-waters, soakages from cesspools, etc., gave $N : C = 1 : 3.126$. In sewage after filtration through soil the proportion of N to C rose from $1 : 1.8$ to from $1 : 4.9$ to $1 : 7.7$. Evidently, therefore, the ratios of N to C "in soluble, vegetable, and animal organic matters vary in opposite directions during oxidation—a fact which renders more difficult the decision as to whether the organic matter present in any given sample of water is of animal or vegetable origin."

All nitrogenous organic matter, whether of vegetable or animal origin, yields more or less ammonia when boiled with a strongly alkaline solution of permanganate of potash, and the ammonia so yielded by potable waters is called "albuminoid," or "organic" ammonia. The proportion of nitrogen in the ammonia so yielded to the total nitrogen in the organic matter is unfortunately not constant; but the chemists to the Massachusetts Board of Health believe that when the process is performed as in their practice, about one-half of the nitrogen is converted into ammonia. Albuminoid substances of animal origin contain about 16 per cent. of nitrogen, but vegetable matters contain very much less; hence the amount of "albuminoid" ammonia is no index to the amount of organic matter present in the water. Mr. Wanklyn, who devised this process, considers that undeniably contaminated waters always yield an excessive amount of albuminoid ammonia (over .10 part per million); usually with much free ammonia (over .08 part per million).

If the albuminoid ammonia distils over very slowly and is in excess, but the water contains little free ammonia and very small quantities of chlorides, Mr. Wanklyn considers this an indication that the contaminating matter is of vegetable origin. He adds: "The analytical characters, as brought out by the ammonia process, are very distinctive of good and bad waters, and are quite unmistakable." The generally accepted opinion, however, is that no reliance can be placed upon these determinations taken alone, and in the *Massachusetts State Board of Health Report* for 1890 there is quoted, as an example, the results of the analyses of certain of the Boston water-supplies. Reservoir No. 4 is known to contain the purest water, but the average "albuminoid ammonia" during two years was .26 per million. The water of the Mystic Lake is the worst of the Boston waters, since it contains both sewage and manufacturing refuse; yet during the same period the average albuminoid ammonia was exactly the same as in the purer water. In the table appended many other examples will be found of the erroneous conclusions which may be drawn from a too implicit reliance upon the determination of the ammonia yielded by distillation with alkaline permanganate.

Forschammer devised a process for the estimation of the amount of oxygen required to oxidize the organic matter in water. This method, as improved by the late Dr. Tidy, has become very popular, and many attempts have been made to render the results comparable with those obtained by Frankland's process, in which

the amount of organic carbon and nitrogen is ascertained by combustion, but with only partial success. The results, when compared with those obtained by the "albuminoid ammonia" process, prove that there is no relation between the amount of ammonia yielded by a water when distilled with an alkaline solution of permanganate of potash, and the amount of oxygen absorbed when the water is digested with an acid solution of the same salt. This process tells us little or nothing of the nature of the polluting material; it does not even distinguish between organic matter of vegetable and animal origin, and it affords us no evidence of the amount of such substances present. The presence of certain bodies of mineral origin (sulphuretted hydrogen, nitrites, the lower oxides of iron, etc.) also cause absorption of oxygen, and unless great care is taken to ascertain the absence of these, or to ascertain the exact amount of oxygen consumed by them if present, serious errors may be introduced. When these corrections are made the oxygen process is still open to all the objections which have been urged against the albuminoid ammonia process. It may condemn a perfectly harmless water as polluted, and pass as of good quality a water of most dangerous character. The following table was devised by Drs. Tidy and Frankland:

AMOUNT OF OXYGEN ABSORBED BY 1,000,000 PARTS OF WATER.

	Upland Surface Water.	Water other than Upland Surface Water.
Water of great organic purity ...	Not more than 1.0	Not more than .5
„ medium purity	„ „ 3.0	„ „ .15
„ doubtful purity	„ „ 4.0	„ „ .2
Impure water	More than ... 4.0	More than ... 2.0

When the quality of a water is considered from the biological side instead of the chemical, the absurdity of dividing waters into classes of pure, medium, doubtful purity, and impure, is obvious. A water containing a poisonous amount of typhoid bacilli might upon analysis be brought within any of these classes, according to the quantity and quality of the accompanying impurities. In the analyses given below there are instances of waters coming within Tidy's limit of "great organic purity," yet which proved to be capable of causing disease. I have examined many such waters myself, and have also passed many waters as perfectly safe for domestic purposes, which a mere reference to the above standards would have condemned as doubtful or impure.

Many other special processes for determining whether a water be safe or dangerous have been devised, but inasmuch as they are rarely used, it may safely be inferred that they possess no advantage over those to which we have already referred.

Sir Charles Cameron, in his "Address on Public Health," delivered before the British Medical Association at Bristol (1894), says: "We have not yet determined, in relation to chemical analysis, the line which divides a bad from a good water, nor

do I believe that such a line can be drawn. One that contains very little organic matter may be dangerous to drink, whilst, on the contrary, water with a large amount of organic matter has been drunk with impunity for years. It is unfortunate that there is no accurate chemical method for determining the precise nature of the organic matter found. As a rule, the larger the percentage of nitrogen contained in the organic matter, the more likely is it to have been derived from an animal source. Analysis certainly shows the amount of organic matter present, and although such matter is usually innocuous, it is sometimes the reverse. The greater the amount of organic matter, or rather of albuminoid matter, the greater the chance that it may include infective substance. When we have a water with a very small amount of nitrogenous organic matter, there is no likelihood that typhoid dejections will be present. Under the ordinary conditions affecting our water-supplies, it is improbable that typhoid stools would be mixed with them. They are thrown into water-closets, privies, ashpits, manure heaps, the soil, ditches, rivers, etc. From all these media they may find their way into rivers or wells furnishing potable water, but with rare exceptions there must, under such circumstances, be large quantities of other kinds of organic matter associated with the typhoid excreta, and carried with it into the water. In short, the passage of unmixed excreta into potable water must be a circumstance of rare occurrence. In the ordinary cases of pollution by typhoid or other dejecta, the analysis of water proves its unfitness for use."

The latter statement is directly contradicted by the results of the chemical examination of very many samples of water which have given rise to epidemics of typhoid fever. A reference to the table already referred to will show that many most extensive epidemics have been attributed to the use of waters which chemists of eminence had declared to be of a high degree of purity. In these cases also repeated analyses failed to prove "unfitness for use."

Whilst no single determination will enable the analyst to certify that a water is free from danger, or that it is so polluted as to be dangerous to health, the determination of several constituents may enable him to pronounce it to be polluted and dangerous, but will never justify him in certifying that it can be used absolutely without risk. As the freedom from all dangerous polluting material is the information usually sought from the analyst, it follows that if this cannot be ascertained by analysis, a chemical examination is in most cases quite useless. Where a water is known to be contaminated with sewage, or known to be liable to such pollution, an analysis is superfluous. When we also consider that many sources of supply are only subject to intermittent pollution, and that waters from the same reservoir or from the same well (*vide* Analyses Nos. 24, 25, and 26, 27) may vary considerably in composition, according to the depth from which the samples are taken, the character of the season, etc., it is obvious that the chemical examination of a water is a matter of comparatively trifling importance compared with the thorough examination of the source of the water and a knowledge of its history. Frequently waters are sent for analysis, and the analyst is wilfully kept in ignorance of their source, lest the information should prejudice his report, yet without this knowledge he is not justified in expressing the opinion that the water from any source can be used with safety. In commenting upon a recent paper in which I expressed these views, a writer in

the *Chemist and Druggist* says: "It would seem, therefore, that we are face to face with the question, 'Is water analysis a failure?' It has been so exclusively the province of chemical analysts to pronounce judgment upon domestic waters, and they generally have given so little attention to the large issues attached to analysis, and so very much to sets of standard figures for chlorine, nitrogen, hardness, and so on, that the attack from the medical health side is not unexpected. There has been more wrangling over water analyses than over anything else in chemistry—and for what? Some figure in the second or third place of decimals, probably, and in regard to what this ammonia or that ammonia implies, when a visit to the source of the water and an inspection of the sewage trickling into it might settle everything. That is what Sir George Buchanan and Dr. Thresh advocate." The Royal Commission on Metropolitan Water Supply received evidence proving that waters containing very large amounts of organic matter were drunk continuously by a population with perfect impunity, whilst other waters containing so little organic matter as almost to defy chemical detection had proved, time after time, to be of the most poisonous character. For these reasons they conclude that the water question has passed from the domain of chemistry into that of biology. This, however, is not strictly correct. The biological problems involved in the investigation of water supplies are numerous and complex, and as yet but imperfectly understood. At the present time it is doubtful whether a biological examination really tells us more than a chemical analysis, and very often it cannot tell us as much. The reason will be explained shortly.

With the discovery of the fact that such diseases as typhoid fever and cholera are due to the introduction into the system, not of dead organic matter, but of actual living organisms, faith in the chemical analysis of waters began to be shaken. When still more recently, the actual microbes causing these diseases had been identified, and processes had been devised for isolating them from the multitude of other organisms found in water, it seemed as though the examination of water for sanitary purposes had passed from the domain of the chemist to that of the bacteriologist. The study of the number and character of the bacteria, it was hoped, would enable the biologist to definitely pronounce whether a certain water was capable of causing disease, or whether it was perfectly harmless in character. Up to the present time such hopes have not been realised, and the results of an ordinary bacteriological examination are as likely to be misleading as those of a chemical analysis. The reason for this is not difficult to explain, when the significance of certain of the discoveries made by bacteriologists is thoroughly understood. An enormous number of species of bacteria have already been discovered, although the science is in its infancy. They are almost ubiquitous, abounding in the air, water, and nearly all articles of food and drink. Of this immense variety very few appear to be capable of causing disease; the remainder are perfectly harmless to human beings, whilst many are already known to discharge most important functions in the economy of nature. Upon their presence the fertility of soil in a great measure depends; they break down the dead organic matter into the simpler forms which can be assimilated by the roots of plants. By their action the foul organic constituents of polluted water are converted into carbonic and nitric acids, which, in combination with the mineral

bases, form innocuous carbonates and nitrates. They are, in fact, nature's scavengers, consuming the foul and effete, and producing therefrom food for the living.

[The remainder of this paper, and the discussion which ensued on it and the preceding paper, will be published in our next issue.]

Purification of Streams by Green Plants, by Bacteria, and otherwise.—Researches on this question by Dr. T. Bokorny (*Arch. f. Hygien*, 1894, xx., No. 2) and Duclaux (*Ann. Inst. Pasteur*, 1894, viii., pp. 117-127) are abstracted in *Royal Micro. Soc. Journ.*, 1894, p. 714. Bokorny remarks on the well-known fact that green plants can assimilate organic substances, and the apparent certainty that aquatic bacteria play a very important part in the purification of water containing a considerable quantity of organic matter; and then recounts his own experiments, chiefly with algæ, and especially with diatoms. Volatile fatty acids, amido-acids, indol, skatol, phenylacetic acid, urea, etc., were exposed in suitable solutions—(a) with free access of light, and (b) with exclusion of light—to the action of algæ. The results showed that algæ were capable of using as nutriment volatile fatty acids, such as butyric and valerianic, and also glucose, leucin, and tyrosin. Even urea, when properly diluted, could be assimilated. It seems clear, therefore, that many organic impurities that are drained into rivers are destroyed by water-plants as well as by bacteria.

Duclaux's work concerns the purification of river-water by bacteria, and its "spontaneous purification," only. After alluding to the condition of the Seine above Paris, and the quantity of impurities deposited in the river from the city, he shows that lower down the river has again become practically pure. He quotes Prausnitz's researches on the water of the Isar, which, when entering Munich, contained 305 germs per c.c., but seven kilometres below the city 12,600 per c.c.; whilst thirty-three kilometres below Munich (eight hours' flow) the number of germs had been reduced to one-sixth. The causes of this purification, according to Duclaux, are both physical and vital—particularly notable among the former being the action of the ground-waters, which mingle with the stream along its course, and which influence purification, partly from their lower temperature in summer, partly from their chemical composition. Another prominent physical mode of purification is the deposition of floating particles, all of which, on account of their density, tend to fall to the bottom and drag down other matters with them, this action being aided by the molecular adhesion of water and the particles; but the purification is effected more surely when an organic or mineral precipitate is formed than when these bodies are left simply to the action of gravity or of molecular adhesion. The principal agent in the purification is, however, the microbe; the rôle of the physical agencies being subsidiary thereto.

F. H. P. C.

On the Micro-Chemical Examination of Quinine. H. Behrens. (*Recueil des Travaux Chim. des Pays-Bas*, xiii., 1, pp. 1-12.)—For the detection of impurities in quinine a series of crystallizations is employed, and the form of the crystals examined under the microscope :

1. *Crystallization of Quinine Bisulphate*.—From 0.05 to 0.2 grammes of the impure sample is warmed with two parts of water, and sufficient H_2SO_4 to retain three-quarters in solution, and crystallized at as low a temperature as possible. If less than two-thirds of the solid taken be obtained, the mother liquor must be slightly concentrated on the water-bath and cooled. The crystals obtained are pure bisulphate, while the other alkaloids are left in the mother liquor. The crystals are separated by pressure with a platinum spatula, and washed with several drops of water.

2. *Crystallization of Normal Quinine Sulphate*.—Small quantities of NaHCO_3 solution are added to the mother liquor with warming, and shaking until neutral, leaving the liquid clear. On cooling a large quantity of the sulphate is deposited in long needle-shaped crystals, which are separated from the mother liquor as before.

3. *Second Crystallization of Normal Sulphate*.—The neutral mother liquor is evaporated to dryness over H_2SO_4 , or over a water-bath, larger crystals being obtained by the former process. The crystals dissolve very slowly in cold water, while the sulphates of quinidine, cinchonine, and cinchonidine may be extracted by adding several drops. A small quantity of this liquid may be warmed, a drop of NaHCO_3 solution added, and the crystals formed examined. Lumpy crystals or rhombs point to the probable presence of quinidine, short needles to cinchonine and cinchonidine, while a powdery deposit, changing on boiling into microscopic drops, denotes a considerable residue of quinine. In the last case the quinine should be removed by precipitation with potassium oxalate before proceeding.

4. *Fractional Precipitation*.—Quinidine is first removed by adding a saturated solution of KI, which forms a cloudy precipitate in a few seconds. Cinchonine and cinchonidine are not precipitated by this reagent. After removal of the greater part of the quinidine, fractional precipitation is employed. The liquid, warmed to 40°C ., is stirred with a platinum wire dipped in NaHCO_3 solution, and the deposit examined under the microscope. If rectangular rod crystals of cinchonine are observed on cooling, they should be left a few minutes to develop. If outside these there are needle-shaped crystals of cinchonidine, the whole should be warmed several times to boiling, the evaporated water being replaced. After this, large well-developed crystals of cinchonine may often be found at the edge of the drops. When undoubted crystals of cinchonine have been obtained, the mother liquor is allowed to cool at one side, and the cinchonine crystals fixed to the glass washed with a drop of water. A second precipitation is then made in the same way, when mixed crystals are often obtained, some of them being the characteristic needles of cinchonidine. Only in the presence of a large quantity of cinchonine is a third crystallization necessary.

The author employed the method successfully in the examination of a mixture consisting of 0.1 gramme impure quinine sulphate, containing 0.5 per cent. cinchonine, 0.3 per cent. cinchonidine, and 0.2 per cent. quinidine.

C. A. M.

The Analysis of Varnishes. Parker C. McIlhiney. (*Jour. Amer. Chem. Soc.*, 1894, 348.) After criticising the various methods which have been hitherto proposed for the separation and estimation of the turpentine and other volatile constituents of a varnish, the author proposes the following. It is based on the well-known fact that on distilling a mixture of two immiscible liquids the boiling-point is lower than that of the more volatile constituent. On distilling together 100 c.c. of water and 5 c.c. of turpentine, the first 95 c.c. of the distillate contains all the turpentine, which readily separates from the water. Experiment showed that 90 c.c. of water either dissolves or holds in suspension about 0.3 gramme of turpentine. On these principles the following process was devised: Twenty-five grammes of varnish are weighed into a flask of 400 c.c. capacity, in which has been placed a piece of granulated tin or its equivalent to prevent bumping, and 10 c.c. water. The contents of the flask are now submitted to distillation, the distillate being caught in a tapped separator. When from 90 to 95 c.c. of water have come over, the distillation is stopped and the turpentine allowed to separate. Should the contents of the flask still retain the odour of turpentine, more water is added and the distillation resumed. The separated turpentine is weighed in a tared flask, and a correction of 0.3 gramme for every 90 c.c. of water made. The results obtained were satisfactory. In some experiments salt was added to the water, but no apparent advantage was gained, except when the non-volatile residue was heavier than water. In this case the salt prevented the residue from sinking to the bottom of the flask, and causing bumping by greasing the flask. If it is desired to examine the residue, the remaining water is poured off, alcohol added, this distilled off, and the last traces of alcohol removed by evaporation with a little ether. The residue is not heated above the boiling-point of water; the turpentine is weighed as such, and may be subjected to further examination. For the determination of petroleum products in turpentine, the method of Burton gives the best results. It depends upon the conversion of the turpentine into acids soluble in water by the action of nitric acid, while the other products remain unaffected.

The analysis of the residue, which consists of linseed oil and one or more resins, all more or less altered by heat, presents an extremely difficult problem, which as yet remains unsolved. The author has applied to these residues the same tests as have been applied to oils—viz., the acid figure, the Koettstorfer figure, the iodine and bromine figures, the bromine addition figure, and the solubility in ether, alcohol, etc.* He arrives at the following conclusions: The differences in the solubility between linseed oil and melted resins are but slight and of little value in effecting separations. The Hübl figure is evidently useless as a means of quantitative analysis, for the figures of linseed oil and kauri, the most frequently occurring resin, are almost identical. The difference in the Koettstorfer figure is only about 75, and even supposing that the figures of both oil and resin are accurately known, which is not often the case, it would be difficult to make the analysis so carefully that the percentages of oil and resin would be correct. The acid figure also fails when applied to a varnish, though it proves correct with a mixture of common resin and linseed oil which has not been heated too long. The bromine absorbed is of no more

* A long list of these figures for a large number of resins, and the corresponding ones for raw and boiled linseed oil, are given in the paper.

use than the Hübl figure, for its quantity depends largely on the conditions under which the analysis is performed. As the bromine-addition figure of resin is 0.0 and that of linseed oil 102, this promised to be of some use; but it completely broke down on account of the changes made by the process of manufacture in the properties of oil and resin. Gladding's process for the estimation of common resin in mixtures with fatty acids, when tried on varnish, also failed to give satisfactory results. The author concludes that it is evident the processes used in oil analysis are not adapted to the analysis of varnishes. The oil and resin react upon one another in some way not understood, giving rise to new compounds, and it is necessary that something further should be known as to the nature of these before this analytical problem can be solved.

W. J. S.

The Viscosimetric Examination of Butter. Dr. C. Killing. (*Zeit. für angewan. Chemie*, 1895, *Heft* 4, pp. 102, 103).—Since the publication of his former communications,* the author has examined a large number of viscosity apparatus, and has found that two may give identical times for water at 20°, without doing so for one and the same fat. The latter will only be similar when the body of the pipette, delivery tube, etc., are of exactly the same dimensions. From this it follows that the time taken by water to run out cannot be taken as a standard for fats, and, accordingly, the viscosity number, which depended on this relation, falls to the ground. This, however, makes no difference in the use of the method, but only in the manner of stating the results. Each apparatus, before being sent out, is now standardized for the mean "running out" time of butter as well as for that of margarine, and is thus ready for immediate use.

Since lard has occasionally been used to adulterate butter, the author gives the time taken by it in running out, and also that of beef fat. In an apparatus giving for butter the mean time of 3 min. 43.5 sec., and for margarine 4 min. 19 sec., the following results were obtained:

Lard.			Beef Fat.		
	min.	sec.		min.	sec.
1.	4	29.00	1.	4	35.50
2.	4	26.75	2.	4	33.50
3.	4	30.25	3.	4	31.00
4.	4	26.25			

As these both take a longer time than margarine, they would be detected even more readily than the latter.

C. A. M.

Note on the Adulteration of Commercial Peptones. M. L. Hugounenq. (*Journ de Pharm. et Chim.*, 1885, pp. 179, 180).—Two samples of peptone, examined by the author, gave an abundant reduction with Fehling's solution. The solution of the peptone, when warmed on the water-bath for an hour with a mixture of sodium acetate and phenylhydrazine chlorhydrate, deposited on cooling a magma of needle-shaped crystals, which, after purification by washing and recrystallization from boiling water, melted at 205°C. This melting-point of the osazone pointed to the

* See ANALYST xx., p. 66.

presence of ordinary glucose. But the absence of alcoholic fermentation in the suspected liquid, and the fact that the compound with phenylhydrazine, although closely resembling phenylglucosazone in the form of its crystals, its melting-point, and behaviour towards organic solvents, was yet distinguished by its solubility in boiling water, showed that the substance was probably lactose. This was confirmed by Rübner's reaction, in which, when a dilute solution of lactose is saturated with a hot neutral solution of lead acetate, and ammonia added drop by drop to the boiling solution, the liquid changes to yellow, then to orange, and finally to red. With pure peptone this does not occur, while in the two adulterated samples it did so very markedly. The amount of milk sugar estimated with Fehling's solution was found to be upwards of 40 per cent. in each case.

The Kjeldahl determination of the nitrogen was also very conclusive. A good peptone from the Lyons hospital yielded 12.72 per cent., while in the two adulterated peptones only 8.34 per cent. was found.

C. A. M.

Chemical Process for Recognizing Horse-flesh. M. Humbert. *Recueil de médecine vétérinaire* (through *Journ. de Pharm. et Chem.*, 1895, pp. 195-198).—In the year 1892 over 20,000 horses were slaughtered in Paris for the purpose of food. Much of their flesh was used in sausages, the vendors of which were compelled to indicate the nature of what they sold. It is thus a matter of great importance to be able to detect horseflesh when mixed with other kinds of meat. The Belgian chemists, Brautigam and Edelmann, have recently shown that it could only be confounded with the flesh of the human foetus and the foetus of animals. For its detection the reaction of iodine on broth obtained from the flesh is used both by these chemists and the author. The latter proceeds as follows: "About 50 grammes of the muscular tissue are cut into small pieces and boiled for an hour with 200 c.c. of water. After cooling, nitric acid is added in the proportion of 5 c.c. to 100 c.c. of broth, and the liquid filtered. To some of the filtrate in a test tube iodine water is added drop by drop, so as not to mix the liquids, and a reddish violet circle will show that horseflesh was present in the original sample. The iodine water is prepared by saturating hot water with iodine. When the reaction is doubtful, it is advisable to replace water by a 3 per cent. solution of KOH. The reaction is more pronounced when the iodine water has been recently prepared. Of ten samples of horseflesh obtained from different dealers in Paris, seven showed the colour very plainly, in two it was less pronounced but still clear, while in the last it was doubtful. In no case was there any coloration with beef, veal, mutton or pork. Beef broth left in contact with the iodine for ten days showed no signs of change. The flesh of the ass also gave a negative result, but with that of the mule the reaction was the same as with horseflesh. A mixture of equal parts of horseflesh, beef, veal, mutton and pork, showed the coloration, but it was less pronounced than with horseflesh alone.

C. A. M.

The Determination of Phosphoric Acid. H. Pemberton, Jun. (*Jour. Amer. Chem. Soc.*, 1895, xvii., pp. 178-181.)—The author cites several precautions to be observed in using his method:

In standardizing the alkali, phenol-phthalein, *not* methyl orange, must be used as

the indicator. Since KOH, as bought, usually contains alumina, the neutral reaction is consequently shown with methyl orange before it appears with phenol-phthalein.

When precipitating the phosphoric acid with the aqueous solution of molybdate, the liquid must be removed from the flame as soon as the boiling point is reached, and *no more heat applied*. The temperature, on adding the molybdate, will then be about 70°, and no water-bath is required.

In order to obtain the precipitate in a granular state, the molybdate solution should be added very slowly from the pipette, the flow being interrupted by closing the end with the finger, and the solution stirred as it goes in.

The washing of the precipitate, which is a difficult matter, is best done on a Hirsch funnel or in a Gooch's crucible, with the aid of a suction pump. A precipitate containing about 50 milligrammes of phosphorus pentoxide is about the largest quantity it is advisable to work on, and where suction is not used, 30 to 40 milligrammes should be the limit. A precipitate containing 40 milligrammes is thoroughly washed on the Hirsch funnel by 300 c.c. of water. C. A. M.

CORRESPONDENCE.

COCOA-NUT OIL IN LARD COMPOUNDS.

To the Editors of THE ANALYST.

SIRS,—In the February issue of THE ANALYST I see that the question of cocoa-nut oil as a lard adulterant has again come up for discussion. As there seem to be very few recorded instances of the presence of this substance in lard compounds, the following example, which I came across a few years ago, may be of interest to your readers.

Dr. Wiley, in his exhaustive report on American lard (United States Department of Agriculture, Div. Chem., Bulletin 13), says: "It is probable that in this country lard is never adulterated with cocoa-nut oil for commercial purposes," and, as far as I know, there have been, so far, no cases reported from this continent.

The sample in question was taken in St. John's, New Brunswick, and was said to have been manufactured by Chase and Deecken, of New York. It was not sent to me until some four months had elapsed since its purchase, and when received it was in a rancid condition. The material was strikingly hard, and had a slightly yellowish colour. It contained 8.44 per cent. of water and 0.57 substances insoluble in ether. The filtered fat had a pale, lemon-yellow colour when cold, a melting-point between 46° and 47° C. and a specific gravity at 99.5° C. (water at 4° C.=1), 0.86449. With the Becchi silver test a walnut coloration was produced, but the reduction took place very slowly. The Hubl number came out 47.8 and the saponification equivalent 259. The crystals deposited from ethereal solution were unlike those from either pure lard or the ordinary lard compounds. Now, though many market samples of lard compound nearly reach the above specific gravity, and the iodine number of a genuine lard may perhaps be as low as 48, yet no possible mixture of lard, stearine, and cotton-seed oil could give both these numbers. I came to the conclusion that the mixture contained about one-third its weight of cocoa-nut oil and a moderate amount of cotton-seed oil.

It is, I believe, claimed that cocoa-nut oil may be so treated as to destroy its characteristic taste and odour. In this instance, though the taste was not apparent at first—perhaps owing to the rancid condition of the sample—the material left a persistent and unmistakable after-taste of cocoa-nut. I may add that it is contrary to my ordinary experience to find more than traces of water in American lard compounds, though I have sometimes found as much as 30 per cent.

EDGAR B. KENRICK.

St. John's College, Winnipeg,
March 6, 1895.